

***Kalbionora palaeotropica*, a new genus and species from coastal forests in Southeast Asia and Australia (Malmideaceae, Ascomycota)**

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Abstract

A new species and genus, *Kalbionora palaeotropica*, is described for a crustose lichen occurring in coastal forests in Thailand, Vietnam, and northeastern Australia. It is morphologically similar to *Malmidea* and *Eugeniella*, but differing in morphological and chemical characters. The single known species in the new genus contains atranorin, zeorin, the stictic acid chemosyndrome and chlorinated xanthones. Morphologically it is characterized by having asci of the *Catillaria*-type, a yellowish brown colour, a granulose epihymenium, dark brown hypothecium, hyaline, 1–3 transversely septate ascospores. Molecular data strongly support a phylogenetic position in Malmideaceae, sister to a clade including *Malmidea*, *Savoronala* and two species currently placed in *Lecidea* s. lat. (including *L. cyrtidia* and *L. plebeja*).

Key words

Lecanorales, lichens, mangroves, taxonomy, tropical diversity

Introduction

Coastal forests in the tropics, especially mangroves, are species-rich habitats and constitute an important part of tropical biodiversity (Donato et al. 2011; Friess 2016). These forests are comprised of unique plant, fungal, and animal species in the interface between marine, estuarine, and terrestrial ecosystems of the tropical and subtropical regions (Hyde et al. 1998; Rangsiruji et al. 2016; Sethy et al. 2012; Stevens 1979). Despite their importance for tropical biodiversity, mangroves are at great risk, with alarming rates of deforestation, especially in Southeast Asia (Friess et al. 2016; Polidoro et al. 2010; Richards and Friess 2016).

Recent studies on the diversity of lichen-forming fungi in Thailand have dramatically increased our knowledge of these organisms in Southeast Asia, with numerous new records and new species discovered in a number of different habitats, including coastal forests (Aptroot et al. 2007; Kalb et al. 2012, 2016a, 2016b; Kantvilas et al. 2010; Luangsuphabool et al. 2016a, 2016b; Naksuwankul et al. 2016; Neuwirth et al. 2014, 2016; Papong and Lumbsch 2011; Papong et al. 2014; Pitakpong et al. 2015; Rangsiruji et al. 2016; Sutjaritturakan and Kalb 2015; Buaruang et al. 2017).

During a recent survey of crustose lichens in mangrove habitats of eastern Thailand, the first author collected a species that appeared undescribed and while superficially resembling the common, pantropical *Lecanora caesiorubella*, showed similarities to the genera *Eugeniella* and *Malmidea*, currently placed in Malmideaceae and Pilocarpaceae, respectively (Jaklitsch et al. 2016; Lücking et al. 2016). This species was also collected by Klaus Kalb in Northeastern Australia, who kindly sent us the material. In addition, revision of material of a record of *Dirina paradoxa* from Vietnam (Joshi et al. 2014) turned out to represent this species as well. A new species and genus is described below based on molecular and phenotypical data.

Material and methods

Morphological and chemical analysis

Specimens were studied from the herbaria F, KoLRI, RAMK, and the private herbarium of Klaus Kalb (Neumarkt). Morphological characters were studied using a Leica Wild M 8 dissecting microscope. Observations and measurements of ascospores were made in water at 630× magnification with a Zeiss Axioscope microscope.

Chemical constituents were identified using high-performance thin layer chromatography (HPTLC), implementing standard methods (Arup et al. 1993; Lumbsch 2002).

Molecular methods

Total genomic DNA was extracted from thallus fragments following the manufacturers' instructions using the ZR Fungal/Bacterial DNA Miniprep Kit (Zymo Research

Corp., Irvine, CA). PCR reactions were performed and primers were used as described previously (James et al. 2006; Schmitt et al. 2010). PCR products were sequenced using an ABI PRISM™ 3730 DNA Analyzer (Applied Biosystems). New sequences were assembled and edited using Geneious v8.1.7 (<http://www.geneious.com>).

RPB2 and *nuLSU* sequences were aligned to each locus independently in the Miadlikowska et al. (2014) alignment (TreeBase no. 156552) using the ‘--add’ option in the program MAFFT v7 (Kato and Standley 2013). For the analysis focusing on Malmideaceae, *nuLSU* and *mtSSU* sequences were aligned using the ‘E-INS-I’ alignment algorithm in MAFFT v7, with the remaining parameters set to default values. A group I intron in the *nuLSU* and present in a limited number of *nuLSU* sequences was not alignable and removed from the data matrix. Ambiguous positions of the *mtSSU* alignment were removed using Gblocks 0.91b (Castresana 2000). Phylogenetic analyses were performed using RAxML-HPG BlackBox 8.2.6 (Stamatakis 2006) and MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) on the Cipres Science Gateway (<http://www.phylo.org>; Miller et al. 2010). The model for each locus used in the phylogenetic analysis was estimated using jModelTest v2.1.9 (Darriba et al. 2012; Guindon and Gascuel 2003). In the ML analysis, the GTR+G+I model was used as the substitution model with 1000 pseudoreplicates. The data was partitioned according to the different genes. Two parallel Markov chain Monte Carlo (MCMC) runs were performed each using 8,000,000 generations and sampling every 1,000 steps. A 50% majority rule consensus tree was generated from the combined sampled trees of both runs after discarding the first 25% as burn-in. The tree files were visualized with FigTree 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Results and discussion

Taxonomy

***Kalbionora palaeotropica* Sodamuk, Leavitt & Lumbsch, gen. et sp. nov.**

Mycobank: MB820208, MB820209

Figure 1

Type. THAILAND, Trat Province: Muang District, Nhong Sa Nho Subdistrict, the route to Nature Education Center Ban Pak Khlong Nam Chiew, on the bark of *Ceriops tagal* (Perr.) C.B.Rob., 2014, *M. Sodamuk* RAMK-24530 (holotype: RAMK; isotypes: F, S).

Diagnosis. Characterized by having asci of the *Catillaria*-type, yellowish brown, granulose epihymenium, exciple consisting of prosoplectenchymatous cells, dark brown hypothecium, hyaline, 1-3 transversely septate ascospores, and the presence of atranorin, zeorin, and the stictic and arthothelin chemosyndromes.

Etymology. The specific epithet refers to the occurrence of the species in the Paleotropics, whereas the genus is named after our colleague Klaus Kalb who has made

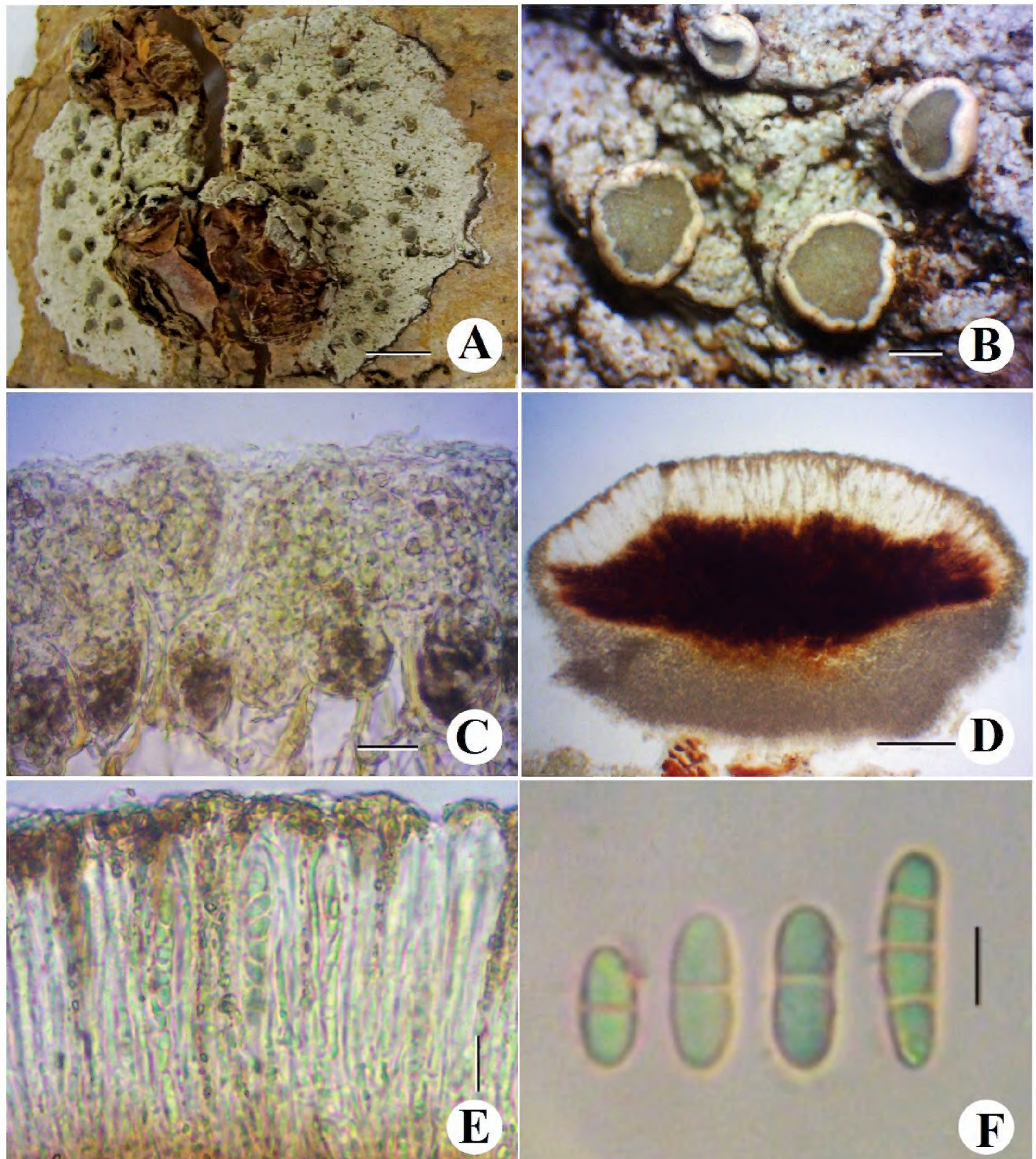


Figure 1. Morphology and anatomy of *Kalbionora palaeotropica*, **A–B** habit **C** cross-section through thallus showing cortex and algal layer **D** cross-section through apothecium showing dark brown hypothecium **E** hymenium, and **F** transversely septate ascospores (holotype). Scale bars: 0.5 cm (**A, B**), 20 μ m (**C**), 0.1 mm (**D**), 10 μ m (**E**), 5 μ m (**F**).

tremendous contributions to our knowledge of tropical lichens and who has been enormously helpful to colleagues in Thailand.

Description. Thallus crustose, corticolous, greenish grey to whitish grey (green fading in herbarium); surface continuous, verruculose, somewhat glossy, prothallus not visible; isidia and soredia absent; corticate, cortex 25–40 μ m thick, covered by a

thin, epinecral layer; photobiont chlorococcoid; medulla indistinct, penetrating into the periderm. Ascomata apothecia, simple, dispersed to crowded, disc plane to convex, grayish green to gray, 0.6–1.6 mm diam.; margin white to whitish grey, thick, entire to flexuous; exciple biatorine, prosoplectenchymatous, incrustated with numerous crystals; hymenium clear, amyloid; paraphyses simple to slightly branched, apically not or slightly thickened; epihymenium distinct, yellowish brown, granulose with numerous small brown crystals, rapidly dissolving in KOH, 3–4 µm thick; hypothecium brown to dark brown, 100–140 µm thick; asci cylindrical, tholus uniformly amyloid, corresponding to the *Catillaria*-type of Hafellner (1984); ascospores 8 per ascus, uniseriate, hyaline, thin-walled, non-halonate, ellipsoid, 1–3 transversely septate, non-amyloid; (8.0)8.9–10.4–11.8(16.0) × (2.5)3.2–3.8–4.4(5.5) µm. Pycnidia not found.

Secondary chemistry. Thallus K+ yellowish, C–, P+ yellow; containing atranorin, stictic acid and zeorin as major constituents, and cryptostictic acid, norstictic acid, peristictic acid, and the chlorinated xanthenes arthothelin and 6-*O*-methylarthothelin as minor compounds (Australian sample analyzed by J.A. Elix).

Distribution and ecology. The new species was found in coastal forests in eastern Thailand, Vietnam, and northeastern Australia (Queensland), growing on bark. It is known only from a few localities but is expected to be more common and potentially overlooked in mangrove forests of Southeast Asia and Australia.

Notes. Morphologically similar is the genus *Malmidea* – some species have similar ascoma morphology and the ascus in this genus also lacks amyloid structures in the thallus. However, this genus can be easily separated by having non-septate, halonate, thick-walled ascospores, and lacking depsidones. Further, molecular evidence suggests that the genera are only distantly related. Another morphologically similar genus is *Eugeniella* and both *Eugeniella* and the new genus also share similar ascospore septation. However, these taxa readily distinguished by the ascus-type (*Byssoloma*-type in *Eugeniella*), the exciple (composed of moniliform hyphae in *Eugeniella*), and the epihymenium (usually indistinct in *Eugeniella*) (Breuss and Lücking 2015; Cáceres et al. 2013a). The new genus might be confused in the field with the superficially similar, common, pantropical *Lecanora caesiorubella* or has been confused with *Dirina paradoxa*, but is readily distinguished by numerous anatomical characters and a different chemistry.

Specimens examined. Australia, Queensland: Daintree National Park, Cape Tribulation, c. 63km N of Mossman, in a dense tropical, coastal rainforest, dominated by *Pandanus* sp., 2008, *K. Kalb* 37355 (hb. Kalb). Thailand, Trat Province: Muang District, Nhong Sa Nho Subdistrict, the route to Nature Education Center Ban Pak Khlong Nam Chiew, on the bark of *Ceriops tagal* (Perr.) C.B.Rob., 2011, *M. Sodamuk*, RAMK—24241, 24242 & 25036 (RAMK); *ibid.*, 2014, *M. Sodamuk*, RAMK—24531, 24532 & 24533 (RAMK); *ibid.*, *Excoecaria agallocha* L., 2011, *M. Sodamuk*, RAMK—25035 (RAMK). Vietnam, Dak Lak Province: Buon Ma Thuot City, Museum, 19 Feb 2013, *Oh & Thanh*, VN130046 (KoLRI).

Phylogenetic analysis

Sequences of *RPB2* and nuLSU rDNA were generated (Genbank nos. KY926780–KY926790) from the type specimen of the new species and added to an alignment used by Miadlikowska et al. with over 1300 representatives in Lecanoromycetes (downloaded from <https://treebase.org> – study no. 156552; Miadlikowska et al. 2014). In a second analysis focusing on Malmideaceae, we aligned nuLSU and mtSSU sequences from three specimens of the new species with all Malmideaceae sequences used in Ertz et al. (2013). Based on the phylogenetic relationship of the new species to other taxa within Lecanoromycetes inferred in this study and published results from Ertz et al. (2013), we selected two species in the genus *Frutidella* and *Miriquidica garovaglii* as outgroups to assess relationships within Malmideaceae.

In our phylogenetic analysis assessing the relationship of *Kalbionora palaeotropica* within the Lecanoromycetes (Suppl. material 1), the type specimen did not cluster with Pilocarpaceae but in Malmideaceae as circumscribed by Ertz et al. (2013). Hence we performed a second analysis focusing on Malmideaceae. In the resulting tree (Fig. 2), the three specimens representing the new species clustered together in a strongly supported monophyletic group, supporting our re-identification of the Vietnamese material [recorded as *Dirina paradoxa* (Joshi et al. 2014)] as belonging to our new species. The new species, which is below described as *Kalbionora palaeotropica*, formed a strongly supported sister-group relationship to a clade including *Malmidea*, *Savoronala*, *Lecidea plebeja*, and *L. cyrtidia*.

In Malmideaceae, *Lecidea plebeja* and *L. cyrtidia* are temperate species occurring in North America and/or Europe and are poorly known. The morphology and distribution of the saxicolous *L. cyrtidia* has been discussed in the literature (Coppins and Muhr 1997; Hertel 1969), and it was suggested that it is closely related to the lignicolous *L. plebeja*, based on shared traits, such as an indistinct thallus, ascus-type, paraphyses with brown apical caps, ascospores of similar dimensions, and similar hypothecium and excipulum. Currently, these two species are poorly understood and additional sampling is necessary to evaluate the relationship of these two taxa. The genus *Savoronala* was recently described to accommodate a single species from coastal *Erica* heathland in Madagascar (Ertz et al. 2013), from which ascomata are unknown. This genus is morphologically characterized by having small, placodioid thalli, sporodochia at the apices of stipes, and brown conidia dispersed with an algal cell. It contains zeorin and usnic acid. The genus *Malmidea* was recently described (Kalb et al. 2011) to accommodate the bulk of corticolous and foliicolous, crustose tropical lichens previously included in the large, polyphyletic genus *Lecidea*, but differing in numerous characters, including the ascus-type (Hafellner 1984). Species in the genus were previously placed in the distantly related, now monotypic genus *Malcolmiella* and includes about 50 species with a thallus usually composed of gonioscysts, usually paraplectenchymatous excipulum, prosoplectenchymatous hypothecium, and an ascus of the *Catillaria*-type, i.e. a tholus with no tubular structures to observe (Breuss and Lücking 2015; Cáceres et al. 2012, 2013b; Kalb et al. 2011, 2012). Species in

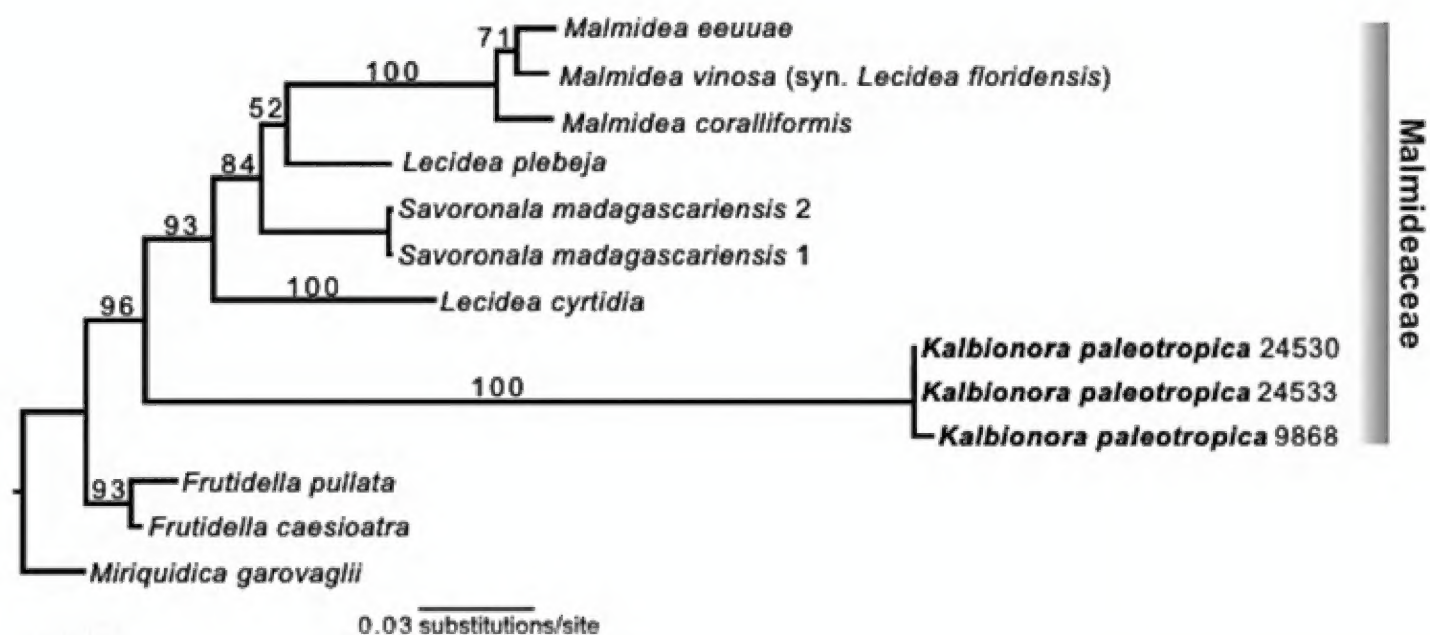


Figure 2. Phylogenetic tree depicting the relationship of *Kalbionora palaeotropica* in Malmideaceae based on mtSSU and nuLSU rDNA sequences. Bootstrap support values above 50% are displayed at nodes.

Malmidea often contain atranorin, sometimes in addition anthrachinones or biphenyls. *Kalbionora palaeotropica* differs morphologically by having a thallus not composed of goniocysts, transversely septate ascospores, and a different chemistry. Molecular data (Fig. 2) support that it is distinct from *Malmidea* and hence a new genus is described here to accommodate this new species.

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Supplementary material I

Phylogenetic tree depicting phylogenetic relationships of *Kalbionora palaeotropica* based on RPB2 and nuLSU rDNA sequences

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Data type: molecular data

Explanation note: Bootstrap support values above 50% are displayed at nodes.

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